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REVIEW

Nucleic acid driven sterile inflammation

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Abstract The etiology of sterile inflammatory conditions is complex and affected by a variety of genetic, environmental and stochastic factors. But despite this overt complexity, progress has been made in elucidating mechanisms underlying disease pathogenesis. An intriguing new finding that has emerged over the past years was the realization that innate immune receptors participate in driving or aggravating disease manifestation. Originally identified as sensors of pathogens and as initiators of antimicrobial immune responses, receptors of the innate immune system recognize a variety of highly conserved microbe associated molecular patterns (MAMPs), including nucleic acids (NAs). While the sensing of DNA and RNA enables detection of a broad range of pathogens this strategy comes at a cost. Indeed, the capacity of NAs to accidentally activate innate sensors significantly contributes to inappropriate responses to self. In this review we will discuss recent findings based on established disease models.

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Abbreviations: DAMP, damage associated molecular pattern; IFN, interferon; IL-, interleukin-; MAMP, microbe associated molecule pattern; NA, nucleic acid; pDC, plasmacytoid dendritic cell; PRR, pattern recognition receptor; RAG, V(D)J recombination-activating protein; STING, Stimulator of interferon genes; TLR, toll-like receptor; TREX1, Three prime Repair Exonuclease 1.

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1. Introduction

During the past decades there has been rapid progress in our knowledge of innate immune recognition of microbes and the critical function this system plays in orchestrating the host adaptive immune response towards pathogens. The conceptual framework that underlies our understanding today dates back more than 20 years, when Charles Janeway proposed the concept of the "pattern recognition theory" [1]. The essence of this theory is that the innate immune system would sense the presence of pathogens via germ-line encoded receptors (PRRs, pattern recognition receptors), which detect highly conserved microbial products (PAMPs, pathogen associated molecular patterns). To be efficient and specific recognition of these PAMPs should fulfill the following criteria: they needed to be common to a wide range of pathogens, unique to pathogens and not subject to rapid adaptive evolution by microbes. This revolutionary idea has proven to be correct, although some aspects required further refinement.

The first and by now also best-documented group of PRRs is represented by the family of Toll-like-receptors (TLRs). So far 10 and 13 functional TLRs have been characterized in humans and mice, respectively, each one exerting a specific function in terms of MAMP recognition [2]. Of note, MAMP (microbe associated molecule pattern) is the more precise acronym for PRR-sensed microbial molecules, given the fact that these ligands are not necessarily confined to pathogens, but can for example also be present in commensal bacteria. TLR4, the first characterized TLR in the mammalian system, was identified as the receptor that responds to Lipopolysaccharide. Other bacterial MAMPs recognized by TLRs include Lipopeptide, Peptidoglycan and Flagellin. All these bacterial MAMPs fulfill the above-mentioned criteria and allow the host to reliably discriminate between self and non-self. However, the discovery of TLRs (TLR3, 7, 8, 9) and other receptors (e.g. AIM2) that can sense microbial nucleic acids (NAs) challenged the concept of traditional MAMPs, given the fact that NAs are in general neither unique nor necessarily specific in their composition for pathogens. Indeed, as such it has soon become clear that endogenous NAs can also effectively activate PRRs.

Along these lines, we know today that a variety of endogenous ligands can potentially activate PRRs and in analogy to MAMPs this class of ligands is commonly referred to as DAMPs (damage associated molecular patterns). DAMPs are often inconspicuous molecules that are normally sequestered within certain cellular compartments and thus invisible to the host's PRR system. Tissue stress or destruction leads to uncontrolled release of these DAMPs and thereby facilitates access to PRRs (e.g. NAs). At the same time, endogenous molecules can also be made visible to the host PRR system due to subtle modifications that arise during tissue stress or damage (e.g. oxidized LDL). Furthermore, DAMPs can also be host molecules that are commonly found within PRR-surveyed compartments under normal conditions, yet can reach critical concentrations that lead to aggregation or crystallization thus initiating PRR activation (e.g. uric acid crystals). Importantly also infection can trigger the release of DAMPs and as such recognition of DAMPs also contributes to antimicrobial immunity.

2. Keeping self-NAs away from PRRs

Given the potential recognition of endogenous NAs as DAMPs, control mechanisms must exist that constrain overt immune reactions towards endogenous RNA and DNA. Along these lines, rapid clearance of potentially harmful self-ligands represents one example of a very important regulatory process. Indeed – as it will be illustrated in greater detail below – degradation of self DNA represents a key checkpoint in maintaining homeostasis and preventing accidental activation of DNA sensors. Another major control mechanism bases on the compartmentalization of innate receptors within certain cellular niches. This latter principle is very well appreciated in the case of TLRs, which are expressed in endosomal compartments of specialized immune cells, such as DCs and macrophages. This class of PRRs is mainly responsible for cell-extrinsic NA recognition and as such detects NAs within engulfed material. But also intracellular sensors at least partly follow the compartmentalization theorem. For instance detection of "foreign" DNA by the inflammasome sensor AIM2 occurs in the cellular cytoplasm carefully separated from endogenous sources of DNA by the nucleus membrane. Nevertheless, sensing NAs as a means of danger comes with substantial costs, as this strategy bears the potential to induce collateral damage to the host. Indeed, over the past years it has become clear that unsuccessful attempts to eliminate endogenous sources of DNA/RNA can trigger autoimmune or autoinflammatory processes.

3. DNA recognition pathways

Of the PRRs that recognize NAs, the family of TLRs is probably the best documented and by now we have obtained a very precise understanding of their mechanism of action (for a detail review on TLR signaling see [2]). The NA sensors within that family include TLR3 (long dsRNA), TLR7 (ssRNA, short dsRNA), TLR8 (ssRNA) and TLR9 (CpG-DNA). Via the adaptor molecules MyD88 (TLR7, 8 and 9) and TRIF (TLR3), these receptors are biochemically linked to downstream signaling that cumulates in the activation of NF-κB and IRFs to induce up-regulation of antimicrobial effector genes. But while this class of NA sensors plays an important role in sensing a variety of pathogens on the basis of their NAs, the generation of mice lacking TLRs revealed that TLR-independent sensors must exist that complement the TLR-dependent innate responses and contribute to successful antiviral defense.

Historically one of the first reports documenting the immunostimulatory potential of DNA in fact depended on a TLR-independent phenomenon [3]. The activation of a TLR-independent signaling pathway by the intracellular delivery of

DNA was then again re-discovered by the groups of R. Medzhitov and S. Akira [4,5]. Both reports came to the conclusion that transfection of synthetic DNA into various cell types induced up-regulation of type I IFNs via the kinase TBK1 and interferon regulatory factor 3 (IRF3). These earlier observations paved the way for the search for an intracellular DNA sensor, the counterpart to the well-described RIG-I-like helicase system that can detect microbial RNAs in the cytosol.

A major breakthrough in defining the cellular signaling underlying DNA sensing was the identification of STING (stimulator of IFN genes), a transmembrane protein localized to the ER [6]. STING-deficient MEFs, DCs and macrophages are incapable of inducing type I IFN after stimulation with different types of synthetic or microbial DNA (expect poly(dA:dT) – see below) [7]. In addition STING is required for type I IFN induction after infection with a variety of distinct DNA viruses like HSV-1, human cytomegalovirus and *Vaccinia virus*. Mechanistically, upon activation STING translocates from the ER to punctate perinuclear compartments, where it interacts with TBK1 and IRF3. Despite its importance, the direct link between STING and DNA recognition is still uncertain. Of note a recent study demonstrated, that bacterial-derived cyclic dinucleotides directly bind to and activate STING [8]. In this regard, two recent reports highlight a novel mechanism on how STING indirectly senses the presence of DNA without binding to it. J. Chen and colleagues discovered that cytosolic DNA binds to a protein (C6ORF150), now termed cyclic G/AMP-synthetase, which in turn synthesizes the 2nd messenger cyclic G/AMP. Cyclic G/AMP then directly engages STING and induces downstream signaling [51,52].

Moreover, a number of DNA receptors that act upstream of STING have been proclaimed; yet the in vivo relevance remains to be proven. Using a candidate gene approach T. Taniguchi and colleagues identified DAI as a putative DNA receptor in L929 fibroblasts [9]. But subsequent studies found no defects in MEFs or DCs lacking DAI [10]. This aspect exemplifies how functional redundancy and cell type specificity may render the identification of DNA sensor(s) more complicated. Other candidate receptors include IFI16 (IFI204 in the murine system), DDX41, LRRFIP1 and Ku70 that are implicated as direct sensors of DNA or facilitators of type I IFN induction, respectively (reviewed in [11]). Future studies with knockout mice and cells will be the definitive test of the function of these candidates in mice and humans and hopefully will provide a clearer picture of the "DNA-interferon" field. One clear exception to the generalization of STING for DNA responses is the cellular responses towards the synthetic DNA mimetic poly(dA:dT). Curiously, it represents the only DNA analog that is capable of inducing type I IFN in HEK293T cells, a cell type that lacks STING. In 2009 we and others demonstrated that transfected poly(dA:dT) is transcribed by RNA polymerase III to generate a triphosphate RNA molecule [12,13]. This in turn can activate intracellular type I IFN signaling via the RNA sensor RIG-I, thus illustrating an indirect mode of DNA recognition.

All the pathways mentioned so far contribute to transcriptional up-regulation of pro-inflammatory genes and type I IFN, yet the presence of intracellular DNA in macrophages and DCs triggers additional cellular responses – namely the activation of the inflammasome. In 2009 the PYHIN protein AIM2 was identified as the missing link between DNA sensing and inflammasome activation [14,15]. Once DNA is bound by AIM2, inflammasome assembly eventually leads to cleavage of procaspase-1, a protease required for processing and secretion of pro-IL-1β and other cytokines. The defect of AIM2-deficient mice in mounting an immune response towards *Vaccinia virus*, *Listeria monocytogenes* and *Francisella tularensis* underscores the physiological relevance of AIM2 for pathogen recognition [16,17].

In parallel with efforts to understand the molecular mechanism underlying the activation of innate immune response towards DNA, attention has been paid to factors that antagonize these pathways by degrading DNA of self and foreign origin. Landmark studies by S. Nagata, R. Medzhitov and coworkers demonstrated that the endonuclease DNase II alpha and the 3′–5′ exonuclease TREX1 (DNAse III) are important negative regulators of the intracellular DNA recognition pathways [18,19]. As will be discussed in the following sections, deletion of these enzymes has deleterious effects with mice succumbing to severe inflammatory responses. While we focus here on the role of DNA recognition pathways for the pathogenesis of autoreactivity, similar mechanisms may be in place for other cytosolic sensors – namely RNA or RNA/DNA hybrids – and are likely to emerge as our understanding of these pathways increases.

4. Autoimmunity vs. autoinflammation

Conceptually, overshooting immune reactivity against self is distinguished into autoimmune and autoinflammatory disorders based on the involvement of the adaptive immune system. Autoimmune diseases are defined as an abnormal response of B cells and/or T cells towards endogenous antigens, which in turn leads to self-directed immunity and eventually presents as either localized tissue damage or as a systemic disease. While breaching of self-tolerance through misled adaptive immunity is recognized as the central event of inducing autoimmunity, autoimmune processes are always tightly connected with the innate immune system and might even be triggered by it. For example, based on their important immunoregulatory function, we know today, that the PRR-triggered production of type I IFNs takes center stage in the pathogenesis of several autoimmune disorders. Alterations of the type I IFN system are typically found in patients with a variety of autoimmune disease such as systemic lupus erythematosus (SLE), Sjörgen's syndrome or type I diabetes mellitus [20]. Through their effects on B cells, type I IFNs contribute to plasma cell differentiation and enhanced autoantibody production. In addition, type I IFNs act as a survival signal for T cells and promote generation of memory and effector T cells. The pathologic consequence of type I IFN production in SLE, for instance, is revealed by disease amelioration in mice with IFNAR1 deficiency [21]. Altogether, type I IFNs have gained increasing attention as key cytokines in the development of several autoimmune disorders, yet the PRR-pathways that induces type I IFN are sometimes less clear.

In contrast, autoinflammatory disorders are sterile inflammatory conditions in the absence of autoreactive T cells or autoantibodies. The first monogenetic cause of such a disease, which later also coined the now common term "autoinflammation", was identified in 1999. D. Kastner's group at the NIH identified a mutation in the gene encoding for tumor necrosis factor receptor 1 (TNFRSF1A) as the cause of a rare autosomal dominant hereditary periodic fever syndrome (now

called TRAPS). Shortly after, the cause for the more common Mediterranean fever syndrome was pinpointed to mutations in the protein PYRIN. Subsequently, additional genetic loci for autoinflammatory diseases were identified, including gain of function mutations in NLRP3, which was later to be identified as a key sensor for DAMP molecules within the inflammasome pathway. The identification of the key signaling components operational in these disease entities, e.g. linking NLRP3 to caspase-1 and IL-1β maturation, allowed classifying autoinflammatory diseases due to yet unknown or polygenic causes. In fact, in the case of NLRP3, several endogenous DAMPs such as uric acid crystals have been shown to trigger inflammasome activation and subsequent IL-1β production and thus these settings can also be understood as "ligand-dependent" autoinflammation. In line with a pivotal role of IL-1β in the pathogenesis of multiple autoinflammatory syndromes, blocking IL-1β results in remarkable serological and clinical responses in patients [22]. Together these findings established a clear link between a cellular innate signaling pathways, in particular the inflammasome-mediated activation of innate immunity, and the roots of autoinflammatory disorders.

5. The autoimmunity—autoinflammation continuum

The insights into the mechanisms underlying autoimmune and autoinflammatory syndromes have re-shaped our perception of immunological diseases. A concept that had been proposed and is referred to as "immunological disease continuum" [23], suggests, that all sterile inflammatory conditions can be accommodated within a continuous spectrum from pure autoimmunity to pure autoinflammation according to the relative contributions of both innate and adaptive mechanisms to a particular disease. In the following, we would like to take this concept as theoretical framework to consider the roles of NA sensing pathways in paradigmatic experimental models of autoimmunity and autoinflammation. In this respect, we want to discuss model systems in which a genetic mutation causes a prototypical disease by disturbing a key biological process thereby resulting in the accumulation of a NA DAMP molecule. Even though these monogenetic sterile inflammatory conditions are very rare or might even be non-existent in the human system, the associated disease pathologies are usually mechanistically highly informative and can generally be extrapolated to broader disease entities. However, in this context we want to point out that we cannot provide a complete overview of clinical disease entities or murine disease models, yet rather want to highlight disease models that we consider extremes of the above-mentioned spectrum (Fig. 1).

6. Disease models of NA induced sterile inflammation

6.1. Autoimmunity triggered by excess extracellular supply of NA

SLE is a systemic autoimmune disease characterized by the production of antinuclear autoantibodies (ANAs) and accompanied by the inflammatory infiltration of many organ systems. The etiology of SLE is considered to be a combination of multiple genetic and environmental factors, which act in concert to cause multiple and stepwise failures of immune regulation. As it is the case for many complex polygenic autoimmune disorders, the molecular mechanisms leading to disease pathology might not necessarily be the same for all affected individuals. Nevertheless, several independent routes of investigation agree on a central role for NA sensing TLRs in the pathogenesis of SLE. In this regard, much of our knowledge of the pathogenesis of SLE has derived from studying mouse models of SLE, whereas one of the most common models of spontaneous lupus represents the MRL/Mp$^{lpr/lpr}$ mouse strain. These mice develop a SLE-like phenotype characterized by elevated serum levels of autoantibodies and high concentrations of circulating immune complexes, which ultimately leads to immune complex-mediated glomerulonephritis and accelerated mortality [24]. The *lpr* mutation is caused by the insertion of a transposable element within the *F*as gene, which leads to a dramatic decrease in *Fas* expression, thereby resulting in a defect in lymphocyte apoptosis. Notably, mutation in the same gene in humans can cause the so-called autoimmune lymphoproliferative syndrome (ALPS), which is characterized by aberrant expansion of T cells and also the development of an autoimmune phenotype [25].

One of the characteristic signatures of SLE is the occurrence of antibodies specific for NA and/or NA-related proteins. Once complexed with their appropriate antigens, these autoantibodies are thought to have direct pathogenic effects by inducing cell death and promoting tissue damage in target organs. In addition, NA containing immune complexes were also found to be potent mitogens for rheumatoid factor specific B cells via simultaneous engagement of TLR9/TLR7 and the B cell receptor (BCR) [26–28]. The importance of NA-specific TLRs for the pathogenesis of SLE was further corroborated in vivo. Crossing mice deficient in TLR9 or TLR7 to MRL/Mp$^{lpr/lpr}$ mice revealed that in vivo these TLRs were required for the generation of antibodies to DNA- and RNA-containing antigens, respectively, by promoting activation and proliferation of anti-DNA/RNA plasmablasts [29]. In spite of their similar expression and activation profile, however, only TLR7 deficiency ameliorated autoimmune disease, whereas TLR9 even accelerated disease progression. The reason for this discrepancy is currently not known.

Together, these studies established the so-called dual receptor paradigm in SLE [30]: Surface-located BCRs specific for immune complexes, lead to internalization of NA immune complexes and deliver them to intracellular TLR-containing compartments. Besides activating B cells, immune complexes of NA represent potent stimuli for type I IFN production by pDCs [31]. In analogy to B cells, pDCs are also highly responsive to NA containing immune complexes via a dual activation mechanism: Interaction with FcγRIIa (CD32) at the cell surface leads to efficient internalization of immune complexes and delivery to endosomal compartments where the NA sensing TLR7 and TLR9 reside [32]. In turn, type I IFN by pDCs further fosters the pathogenesis of autoimmunity by facilitating autoantibody production by B cells and via up-regulation of a plethora of IFN-stimulated genes, which in turn lower the threshold for consecutive stimulation by innate sensors. By that means an important intrinsic checkpoint, which under normal circumstances regulates avoidance of self-NA recognition

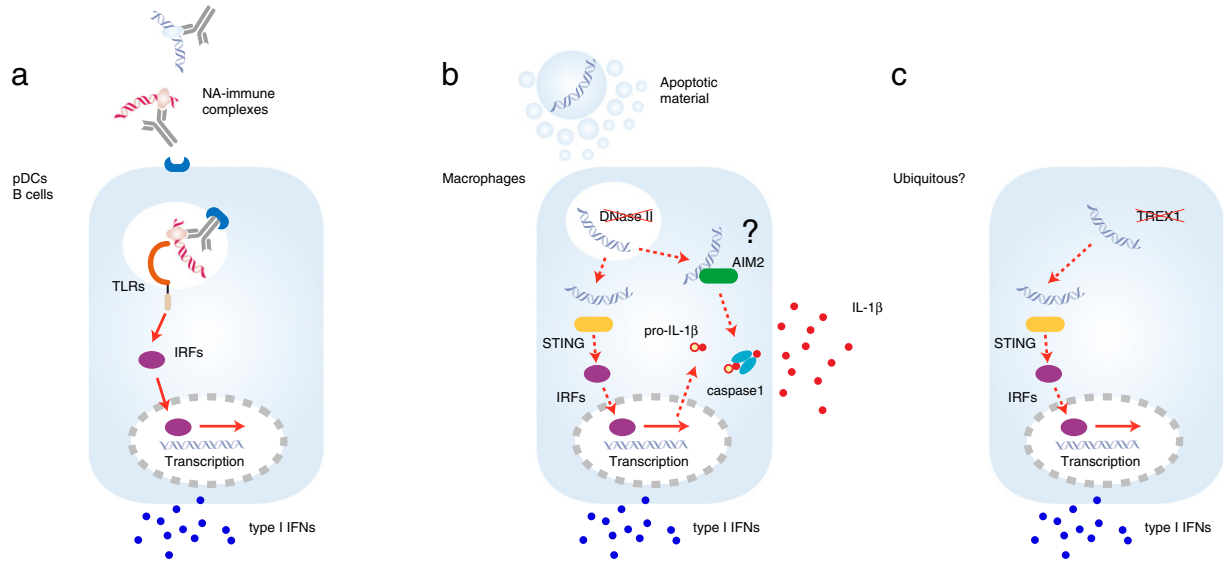

Figure 1 Nucleic acids drive sterile inflammation through multiple mechanisms. a, Once captured by autoantibodies extracellular self nucleic acids gain access to endosomal TLR7/9 in B cells and pDCs and trigger cell proliferation, antibody production and type I IFN secretion, respectively. b, Under certain pathologic conditions, self nucleic acids may accumulate intracellularly. Apoptotic cells are internalized by macrophages and in turn delivered to lysosomal compartments. In the absence of lysosomal DNase II alpha, self DNA is released into the cell cytoplasm and stimulates STING-dependent production of type I IFNs and pro-inflammatory cytokines. At the same time, activation of the inflammasome pathway may occur. c, Endogenous sources of DNA (reverse transcribed retroelements) accumulate in the cytoplasm due to defective clearance by Trex1 (DNase III). Due to its ubiquitous expression, the latter scenario is not restricted to specialized immune cells but instead occurs also in non-hematopoietic cells.

through sequestration of TLRs, is overcome and endogenous sources of DNA and RNA provide a permanent source of potent immunological triggers. Once established, this process than triggers the fatal circuitry of autoantibody production, immune complex formation and activation of inflammation.

The results discussed so far focused on the effect of NA sensing TLRs once immune complexes have already been formed and the process of autoimmunity has already been initiated. But this mechanism cannot account for the primary event in SLE, as it depends on the pre-existence of autoantibodies and immune complexes. So what are the factors – genetic or environmental – that activate immune responses in first place? An early event in SLE that many groups agree on today is the concept of ineffective clearance of dead cells and the consecutive oversupply of cellular debris, including immunogenic NAs. One hypothesis suggests, that accumulation of NAs in germinal centers acts as survival signal to directly drive the expansion of non-tolerant B cells [33]. As such simultaneous engagement of BCRs and TLRs might be sufficient to ignite the process of autoimmunity in SLE. An alternative model proposes that the initiation phase in SLE is rather mediated by specialized DC subsets, which become activated by apoptotic cells in a TLR-independent fashion [34]. Central within this model is the early production of type I IFNs that control initial events such differentiation of APCs and activation of autoreactive quiescent T cells and B cells. But while SLE may originate in several different ways, the pathogenesis – at least in mice – clearly depends on the activation of APCs via endogenous sources of nucleic acids. An interesting so far unresolved question is whether and to what degree intracellular NA receptors might participate as well (see below).

6.2. Autoinflammation induced by accumulation of intracellular DNA — the DNase II alpha model

The elimination of dead cells by phagocytic cells is a crucial event to maintain normal tissue function. This task is being accomplished by tissue resident phagocytes and works by detection, engulfment and degradation of cellular contents into their basic biochemical components. Naturally, also endogenous DNA is present within cellular remnants and constitute a potential source of DAMPs within phagocytic cells. Under physiological conditions, however, a set of lysosomal enzymes – including DNase II alpha – keeps the amount of DNA beneath a certain threshold and as such avoids activation of innate sensors. This function of DNase II alpha is nicely exemplified in the course of definitive erythropoiesis, which takes place in so-called "blood islands" within the liver. During that process macrophages engulf expelled nuclei from erythroid precursors as they mature into enucleated erythrocytes. Blood-island-macrophages from DNase II alpha deficient animals are incapable to degrade DNA after phagocytosis and activate innate immune signaling pathways. This in turn culminates in the production of cytokines including type I IFNs and TNFα [18]. As a consequence of unabated cytokine production, mutant embryos from Dnase II alpha−/− mice suffer from severe anemia and ultimately die in utero. Crossing Dnase II alpha−/− mice with mice lacking the IFN receptor (IFNAR1), or IRF3 and IRF7 abrogated type I IFN production [18,35]. Strikingly, in each case, the mortality and anemia observed in Dnase II alpha−/− embryos were nearly completely rescued. But irrespective of blocking type I IFNs and prolonging survival of the animals, macrophages of Dnase II

alpha−/− animals are still activated and capable of secreting high amounts of TNFα and other pro-inflammatory cytokines. As a consequence of the persisting systemic inflammatory condition these mice develop a form of chronic polyarthritis at 2–3 months after birth [36]. Likewise, conditional inactivation of Dnase II alpha after birth promotes the development of arthritis. Histologically affected joints show cartilage erosion, bone destruction and pannus formation. Moreover, inflamed joints are infiltrated with lymphocytes, neutrophils and macrophages, a finding which is paralleled with high levels of cytokines (TNFα, IL-6, IL-1β) and chemokines. In addition Dnase II alpha−/− mice exhibit elevated levels of matrix metalloproteinase (MMP)-3, anti-cyclic citrullinated peptide (CCP) antibody, rheumatoid factor, which are all diagnostic hallmarks of RA. These changes in serum parameters are accompanied with swelling of joints, which first manifests in the periphery. Exploring the details of the mechanism underlying the pathogenesis of arthritis caused by DNase II alpha deficiency revealed some interesting points [37]. First, as judged by the analysis of bone marrow chimera experiments, the selective deficiency of DNase II alpha within the bone marrow was sufficient to initiate arthritis. Indeed, macrophages within afflicted joints did not contain more DNA. Secondly, excessive cytokine production within afflicted joints seemed to rely on positive feedback regulation. Genetic or pharmacological inhibition of any of the key inflammatory cytokines (TNFα, IL-6, IL-1β) significantly diminishes the others as well. In addition, injection of anti-TNFα or anti-IL-1β after disease onset was found to ameliorate arthritis and reduce joint damage in this model. Thirdly, arthritis even develops in the absence of adaptive immune responses (RAG2−/− background).

From these studies a picture emerges, that places DNA-induced cytokine production in the bone marrow and spleen at the etiopathogenic center of one form of polyarthritis. Once released into the circulation macrophage-derived TNFα, IL-6 and IL-1β are involved in the initiation of the inflammatory process in the joints. Local intraarticular production of the same cytokines via positive feedback regulation then establishes a "cytokine storm", which further sustains and propagates the arthritis. This pathogenic process includes proliferation of synovial cells and macrophages as well as recruitment of neutrophils, monocytes and lymphocytes, which finally catalyze joint destruction. The exclusive affection of joints by this systemic pro-inflammatory state appears puzzling. Indeed, it cannot be ruled out that other tissues are also directly or indirectly affected, yet the joints appear to present the most overt phenotype in this model. The finding that lymphocytes are dispensable for the development of arthritis points towards innate mechanism as major factor for disease initiation and progression and therefore places the DNase II alpha−/− model to the autoinflammatory side within the immunological disease spectrum.

The DNase II alpha knockout model shares some overlap with the human disease entity rheumatoid arthritis (RA), one of the most common autoimmune disorders worldwide (prevalence estimated 0.5–1%) [38], yet also differs in several important points. RA is a complex polygenic disease that is also associated with several environmental factors. Immunological studies, identified genetic risk loci and successful treatment modalities show that RA is an autoimmune disease, clearly involving the activation of the adaptive immune system. It is suspected that chronic activation of the innate immune system perpetuates the disease and that neoantigens formed by protein citrullination constitute the main target for the adaptive branch of the immune system. DNA as "the" main inflammatory inducer, as it is the case in the DNase II alpha knockout model, has yet unknown relevance to RA. Indeed, as pointed out by Nagata and colleagues, the DNase II alpha−/− model rather shares strong parallels to systemic juvenile idiopathic arthritis (SJIA, also known as Still's disease) [37], for which accumulating evidence suggests that it can be seen as an autoinflammatory condition [39]. Considering the DNA receptor system(s) that mediates this phenotype several candidates might be responsible. Of note G. Barber and colleagues just recently provided compelling evidence showing the involvement of the STING-pathway in mediating inflammation-related embryonic death in DNase II alpha deficient mice [40]. DNase II alpha−/−, STING−/− double knockout embryos exhibited no signs of anemia and were rescued from lethality compared with DNase II alpha knockout embryos. This phenotype paralleled with significantly reduced levels of type I IFN up-regulation and induction of pro-inflammatory cytokines such as TNFα in DKOs versus DNase II alpha−/− embryos. Interestingly, absence of STING also prevented development of self-DNA induced polyarthritis. Of note, in addition to innate gene expression DNase II alpha−/− macrophages show activation of inflammasome markers such as caspase-1 activation and IL-1β release suggesting a plausible role for the AIM2 inflammasome in the pathogenesis of the disease [37]. Additional studies aimed to precisely separate the sensing identity/identities for undigested DNA will certainly provide an important piece to the puzzle.

6.3. Aicardi-Goutières syndrome — an autoimmune disease triggered by intracellular accumulation of NAs

An alternative, yet, complementary mechanism that constrains intrinsic DNA accumulation bases on the 3′→5′ DNA exonuclease TREX1 (*T*hree prime *R*epair *Ex*onuclease 1, originally designated DNase III). Close amino acid sequence homologies to E. coli proofreading exonucleases and the ability to remove mismatched 3′ terminal bases first suggested that TREX1 might serve an editing function during DNA replication [41,42]. However, TREX1−/− mice did not show increased spontaneous mutation frequency or cancer incidence in vivo, as one would expect if TREX1 was obligatory for editing during DNA repair or replication [43]. Instead, TREX1 deficiency resulted in dramatically reduced survival along with cardiomyopathy and circulatory failure due to the development of an inflammatory myocarditis. But while the link between TREX1 and inflammation remained obscure, following studies in humans further supported hyperactivation of the immune system in the absence of functional TREX1. Initially mistaken for congenital viral infection, we know today that the neurodegenerative disease Aicardi–Goutières syndrome (AGS) is a hereditary disorder based on mutations within at least five independent loci (reviewed in [44,45]). Next to TREX1 (mutated in patients of the AGS1 group) mutated versions of RNASEH2B (AGS2), RNASEH2C (AGS3), RNASEH2A (AGS4) and SAMHD1 (AGS5) constitute different genetic etiologies of AGS. While varying in the severity and outcome of the disease, all mutations cause similar symptoms primarily

affecting the brain and the skin. Clinically AGS presents as an early onset progressive encephalopathy caused by calcifying vasculitis. Similar to intrauterine viral infections excessive type I IFN production is being recognized as a central pathogenic event in driving the phenotypes observed in AGS. In fact, increased levels of IFN-α and lymphocytosis in the CSF are considered as reliable and indicative markers of AGS in the absence of viral infection. Occasionally patients show also extraneurological manifestations, including bouts of mild fever or so-called chilblain skin lesions, a cold-induced cutaneous form of lupus erythematosus. Mutations within TREX1 have also been associated with a familial form of chilblain lupus and also in patients with SLE, which implies that AGS and certain types of SLE are different phenotypes elicited by similar pathomechanism [46,47].

Recently, several groups have provided a crucial insight into the mechanism that underlie the pathogenesis of AGS and have explained how the absence of TREX1 triggers type I IFN production. Initially purified from cell nuclei, TREX1 predominantly localizes to the ER, presumably by binding to the ER membrane or by interacting with ER-resident proteins [19]. Consistent with a preferential affinity for single-stranded DNA, Yang et al. reported that TREX1−/− cells accumulate discrete ssDNA species in the cytosol [48]. While the authors initially ascribed the arising ssDNA species as byproducts from defective DNA replication, Stetson et al. identified endogenous retroelements as the source of aberrant DNA accumulation in TREX1−/− cells. Regardless of the origin of DNA it is now clear that abnormal accumulation of intracellular DNA in TREX1−/− cells triggers the above described STING–TBK1–IRF3 signaling axis and induces transcriptional up-regulation of type I IFNs. TREX1−/− mice lacking individual components of this cascade are protected from developing lethal autoimmunity. Moreover, genetic ablation of the type I IFN receptor (IFNAR1) completely prevented tissue damage and mortality in TREX1−/− animals, which is consistent with IFN signaling being a crucial upstream event in disease initiation and progression. More recently, Gall and Stetson explored the steps of autoimmunity in the murine TREX1 model in more detail [49]. Utilizing an in vivo reporter system to track type I IFN activity in combination with several genetic crosses this study provides important novel aspects in dissecting the pathway linking TREX1 deficiency to lethal autoimmunity. As expected from previous studies type I IFN production is already apparent in utero and does not depend on the presence of lymphocytes (RAG2 deficiency does not impact on overall interferon production). Instead, a tissue-restricted IFN response first develops in non-hematopoietic cells, defining this cellular compartment as the initiator of the disease process. But despite elevated production of type I IFN found in TREX1−/− on a RAG2 background, these mice are still resistant to develop autoimmunity. These findings suggest, that type I IFNs do not confer the pathology on their own but instead require the presence of lymphocytes and as such activation of the adaptive arm of the immune system. As revealed by a series of elegant mixed bone marrow chimeras, type I IFN signaling favors the expansion and recruitment of autoreactive T cells to the target organ. Assessing the individual role of αβ T cells versus B cells in the disease model revealed, that T cells are necessary and sufficient to drive autoimmunity and that they are essential for the increased mortality due to TREX1 absence. Gall et al. also found that mice that lack B cells exhibited a dramatic increase in lifespan despite no amelioration of inflammation in affected tissues. However B cells possibly contribute to glomerular damage by deposition of autoantibody–antigen complexes. As mentioned above, next to TREX1 mutations within the loci of RNASEH2A, B and C and SAMHD1 contribute to the genetic causes of AGS. However the molecular mechanisms that connect these factors with innate immune activation are yet not understood.

7. Concluding remarks

The innate immune system has evolved multiple sensing strategies, which are called into place to induce an immediate response and thus protect the host against infecting pathogens. The detection of NAs as a hallmark of infection is very efficient, yet challenging as NAs often lack characteristic pathogen features. The evolution of an intricate NA detection system has resulted in a trade-off between self-protection and autoreactive processes. The sum of intra- and extracellular nucleases and segregation of receptors within subcellular niches decrease the likelihood of mounting innate responses towards self NAs. Further, it seems conceivable that additional, so far unnoticed, regulatory mechanisms do exist that limit the sensitivity towards the nucleic acid DAMPs in non-inflamed tissues. In this regard, it is plausible that environmental parameters critically impact on the way NAs are "interpreted" on a cellular level. Since microbial infection is commonly accompanied by severe disturbances of tissue homeostasis, the associated release of additional DAMPs might provide the molecular context that favors responsiveness towards intracellular DNA. Of note, in vitro studies might underestimate the importance of these combinatorial effects.

A paradigm that has emerged over the last couple of years is that the induction of a type I IFN response upon viral infection is completely dependent on the recognition of nucleic acids in non-immune cells. The tight connection between NA sensing and IFN production is also supported by the exclusive expression of only TLR7 and TLR9 in pDCs, the major IFN producing cells in the body. Therefore, regardless of the origin of NA (extracellular versus intracellular), activation of the respective receptors is polarized towards type I IFN production, an important cytokine family in bridging innate with adaptive immunity. This notion also helps to explain why in the context of excess accumulation of endogenous NAs, autoimmunity rather than autoinflammation develops. Of note, the dominant inhibitory activity of type I IFNs on the inflammasome/IL-1β system, might even exacerbate this polarity and shift it towards autoimmunity [50].

Interestingly, the above-described DNase II alpha knockout mouse model – with yet unclear clinical relevance – appears to be an exception to this rule, with disease pathology displaying a clear autoinflammatory component. Along this line, it is likely that the cell type promoting the NA sensing function represents a critical determinant in the type of inflammatory process to develop. Loss of DNase II alpha activity positions disease pathology within the macrophage compartment of the bone marrow, a cell lineage that is well equipped with components of the inflammasome pathway. TREX1 deficiency on the other hand appears to impact on multiple cell types and the involved DNA sensor STING is also ubiquitously expressed. Consequently type I IFN production in

this disease model can arise from multiple cell types. At the same time it has to be considered that besides the local microenvironment, the timing of activation of the innate immune system and the availability of self antigens also play important roles in determining the outcome of a sterile inflammatory condition. In addition, it is important to note that the most common sterile inflammatory conditions in the human system are a result of multiple genetic, environmental and stochastic factors, which of course complicates the formulation of a unifying pathogenesis model. As such, these diseases are usually found somewhere in between the extremes of the autoimmunity–autoinflammation continuum, with both systems playing a pathogenetic role.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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